Art 34

CLAIMS:

1. A method for sequencing DNA, which comprises:

(a) obtaining a target DNA population comprising a plurality of single-stranded DNAs to be sequenced, each of which is present in a unique amount in the same reaction zone and bears a primer to provide a double-stranded portion of the DNA for ligation thereto;

(b) contacting the DNA population with an array of hybridisation probes, each probe comprising a label cleavably attached to a known base sequence of predetermined length, the array containing all possible base sequences of that predetermined length and the base sequences being incapable of ligation to each other, wherein the contacting is carried out in the presence of ligase under conditions to ligate to the double-stranded portion of each DNA the probe bearing the base sequence complementary to the single-stranded DNA adjacent the double-stranded portion thereby to form an extended double-stranded portion which is incapable of ligation to further probes; and

(c) removing all unligated probes; followed by the steps of:

(d) cleaving the ligated probes to release each label;

(e) recording the quantity of each label; and

(f) activating the extended double-stranded portion to enable ligation thereto; wherein

(g) steps (b) to (f) are repeated in a cycle for a sufficient number of times to determine the sequence of each single-stranded DNA by determining the sequence of release of each label.

2. A method according to claim 1, wherein the array comprises a plurality of sub-arrays which together contain all the possible

base sequences, and wherein each sub-array is contacted with the DNA population according to step (b), unligated probes are removed according to step (c), and these steps are repeated in a cycle before step (d) so that all of the sub-arrays contact the DNA population.

3. A method according to claim 1 or claim 2, wherein the target DNA population is obtained by sorting an initial DNA sample into sub-populations and selecting one of the sub-populations as the target DNA population.

4. A method according to claim 3, wherein the initial DNA sample is cut into fragments, each having a sticky end of known length and unknown sequence, which fragments are sorted into sub-populations according to their sticky end sequence.

5. A method according to any one of the preceding claims, wherein each single-stranded DNA is immobilised at one end.

6. A method according to any one of the preceding claims, wherein the label of each probe comprises a mass label, and the quantity of each label is recorded according to step (e) using mass spectrometry after release of the label in step (d).

7. A method according to any one of the preceding claims, wherein the known base sequence is blocked at its 3'OH.

8. A method according to claim 7, wherein the step (d) of cleaving the ligated probes to release each label unblocks the 3'-OH of the extended double-stranded portion according to step (f).

9. A method according to claim 8, wherein the label of each probe is cleavably attached to the 3'-OH of the base sequence.

10. A method according to any one of claims 1 to 6, wherein the base sequence of each probe is unphosphorylated at both 3' and 5' ends and step (f) comprises phosphorylating the 5'-OH of the extended double-stranded portion.

11. A method according to any one of the preceding claims, wherein the predetermined length of the base sequence is from 2 to 6.

12. A method according to claim 11, wherein the predetermined length of the base sequence is 4.

13. A kit for sequencing DNA, which comprises an array of hybridisation probes, each probe comprising a label cleavably attached to a known base sequence of predetermined length, the array containing all possible base sequences of that predetermined length and the base sequences being incapable of ligating to each other.

14. A kit according to claim 13, wherein the known base sequence is blocked at its 3'-OH.

15. A kit according to claim 14, wherein the label of each probe is cleavably attached to the 3'-OH of the base sequence to prevent ligation thereto.

16. A kit accordng to any one of claims 13 to 15, wherein the base sequence of each probe is unphosphorylated at both 3' and

5'' ends.

17. A kit according to any one of claims 13 to 16, wherein the label of each probe comprises a mass label.

18. A kit according to any one of claims 13 to 17, wherein the predetermined length of the base sequence is from 2 to 6.

19. A kit according to claim 18, wherein the predetermined length of the base sequence is 4.

20. Use of a kit according to any one of claims 13 to 19 for a method of sequencing DNA.

add A'

add B'